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IRON ACCUMULATION IN TUNICATE BLOOD CELLS. II. WHOLE BODY AND BLOOD CELL IRON UPTAKE BY *STYELA CLAVA*

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Dedicated to the memory of
Guy C. McLeod

Abstract

Iron accumulation by the tunicate *Styela clava* was investigated. Live specimens did not show uptake of dissolved iron from sea water, although their tunics showed a high affinity for iron adsorption. In partitioning experiments, the tunic of *S. clava* was shown to be impermeable to dissolved iron. The most likely source of iron is particulate matter that tunicates filter through their siphons from sea water.

Iron uptake by *S. clava* blood cells was studied. Iron(II) uptake by the cells was shown to be biphasic. Uptake was shown to be an irreversible process for the time span studied. The metabolic inhibitor 2-deoxyglucose did not inhibit uptake. Citrate blocked uptake. Addition of Ga(III) did not affect uptake, implying that iron(II) does not undergo oxidation changes during accumulation by the blood cells. Uptake did not occur through anionic channels. Iron(III) uptake studies were inconclusive because of the low solubility of uncomplexed iron(III) species.

Introduction

Most ascidians are known for the high content of vanadium in their blood and for their ability to concentrate vanadium from sea water. However some ascidians lack vanadium, but contain high concentrations of iron in their blood (Endean, 1953; Agudelo *et al.*, 1983). The taxonomy of ascidians reflects the separation of species into vanadium and iron containing species (Hawkins *et al.*, 1983). In the suborders Aplousobranchia and Phlebobranchia, the majority of species' blood contains vanadium. In the suborder Stolidobranchia, vanadium is absent and iron is the major metal in the blood.

Vanadium accumulation by vanadium-containing tunicates has been studied in detail, and some of the steps of vanadium transport have been clearly defined. In sea water, vanadium is present as monomeric vanadate, at a concentration of 6×10^{-8} *M* (Ladd, 1974). Kustin *et al.* (1975) determined that tunicates remove vanadate from the aqueous phase of sea water through the alimentary tract. Once it reaches the tunicate's blood, vanadate is transported through anionic channels into the blood cells (Dingley *et al.*, 1981), where it is reduced (perhaps with the aid of tunichrome) to V(IV) or V(III). It is prevented from leaving the cell because of either its positive charge, VO^{2+}, V^{3+}, or complexation to an organic ligand (Macara *et al.*, 1979; Dingley, 1982).

Unlike that of vanadium, the mechanism of iron uptake by tunicates is unknown. The elementary steps of iron uptake are far from clear even in vertebrates, despite the

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fact that iron is a known, essential trace element. In mammals, for example, two types of iron can be absorbed: heme iron, and nonheme iron. Iron in most food materials is predominantly nonheme iron. It is known that absorption is enhanced in the presence of reducing agents or chelating agents which bind ionic iron. Following iron absorption, its transport in mammals involves specific iron transport proteins (transferrins), and special storage units (ferritins) (Bezkorovainy, 1980). Iron accumulation by iron-containing tunicates has the potential to shed light on vertebrate iron uptake, and is a logical extension in the study of metal accumulation in general.

Unlike vanadium, aqueous iron chemistry is very complex because of extensive hydrolysis, and formation of insoluble hydroxide products. In sea water, iron(III) is the main oxidative species if the marine system is controlled by oxygen (*e.g.*, sea water near the coast; surface waters). Iron(II) is the main species if the marine environment is devoid of oxygen (Kester *et al.*, 1975). Since tunicates generally inhabit well-oxygenated waters, we will primarily be concerned with iron(III) chemistry in sea water.

Styela clava Herdman has a high content of iron in its blood cells (Agudelo *et al.*, 1983). Since iron is a common denominator among the species of the suborder Stolidobranchia, we assume iron to be an essential metal for these ascidians. It is not known what role iron plays in ascidian blood cells, although oxygen transport can be excluded as the blood cell function (Agudelo *et al.*, 1982). In the first paper of this series we described the oxidation state and distribution of iron in *S. clava* and two other iron-containing species.

Little has been done to study the method of uptake or function of iron in tunicate blood. The majority of uptake studies carried out with whole animals have been impaired by iron precipitation (Berg, 1982). Most of the work done on iron accumulating tunicates has been done on the iron-plasma protein association (Hawkins *et al.*, 1980; Webb and Chrystal, 1981), and iron tunichrome interactions *in vitro* (Macara *et al.*, 1979). The present study was undertaken to trace iron uptake by iron-accumulating *Styela clava* from sea water to blood cells.

Materials and Methods

Materials

^{55}Ferrous sulfate and ^{55}ferrous citrate were purchased from Amersham Corporation. ^{55}Ferric chloride was obtained from New England Nuclear.

Animals

Specimens of *Styela clava* were obtained from Boston Harbor, at a depth of 1–6 m, and were maintained in a 5°C sea water aquarium. Animals used for uptake experiments varied in size from approximately 5 to 12 cm in height and from approximately 10 to 25 g in weight.

Iron uptake by S. clava *from sea water*

All iron uptake experiments were carried out at 5°C in a plastic container to prevent iron absorption by glass surfaces. *S. clava* specimens were carefully cleaned with a brush and placed in test solutions at time, $t = 0$ (approximately 200 ml of sea water per animal). Aliquots of sea water were removed at timed intervals, for up to 52 hours, and counted using a Beckman LS-100C liquid scintillation counter, to measure the remaining iron in sea water. The rate of uptake was determined from a plot of counts per minute (cpm) *versus* time.

For iron(III) uptake experiments, $^{55}FeCl_3$ was added to sea water along with 1000-fold citrate to prevent precipitation of iron hydroxides. Iron concentrations ranged from 10^{-7}–10^{-6} *M*. Control experiments were run without specimens to insure no iron precipitation. In addition, iron in solution was monitored for several hours before adding the animals. The citrate concentration, added from a neutralized stock solution, never exceeded 5 m*M*. The toxicity of citrate to *S. clava* was checked prior to carrying out the iron(III) uptake studies. Five *S. clava* were placed in 1.66 liters of 0.45 μm filtered sea water in a closed respirometer connected to a YSI oxygen electrode and YSI model 57 oxygen meter. Oxygen consumption was measured for two hours, citrate was then added (total concentration approximately 5 m*M*), and oxygen uptake was measured for an additional two hours.

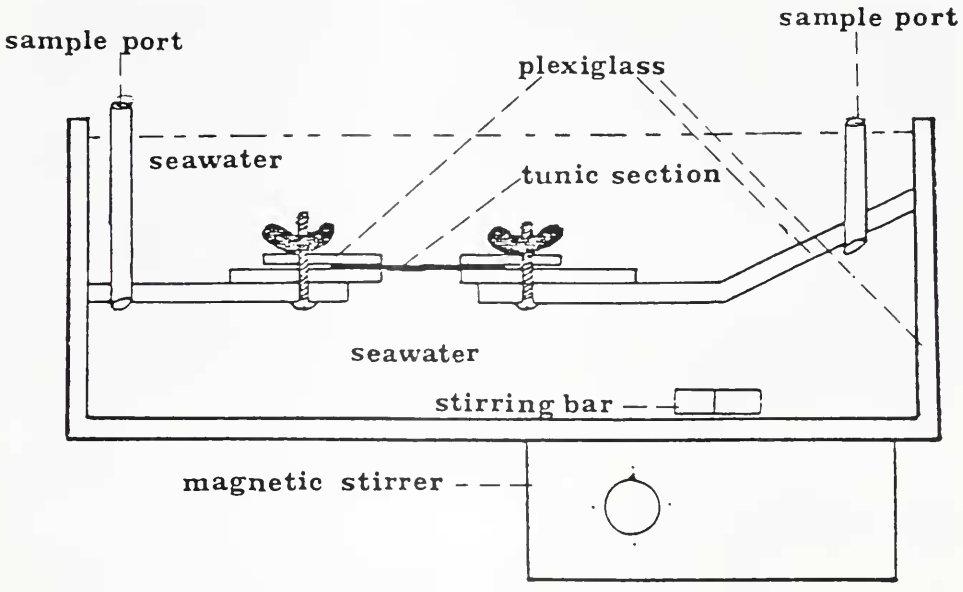
Two additional experiments were conducted to further determine the rate and site of iron(III) uptake. First, the permeability of tunic toward iron was determined using a plexiglass container (Fig. 1) in which pieces of tunic from *S. clava* acted as a partition between two sea water compartments. The outer surface of the tunic (the surface normally exposed to sea water) was exposed to a sea water solution containing radioactive ferric chloride (10^{-7} *M*) and 1000-fold citrate excess. The inner surface of the tunic was exposed to normal sea water (Fig. 1). Sea water samples from each compartment were taken over a two-day period and counted by liquid scintillation.

Second, specimens of *S. clava* were lightly coated with Vaseline to make the surface hydrophobic thereby preventing iron precipitation on the tunic. Animals were then exposed to radiolabeled $FeCl_3$ plus 1000-fold of citrate. Sea water samples were taken at intervals of time over a 52-hour period.

Two iron(II) uptake experiments were carried out. A 20-fold excess of ascorbate was used to maintain the iron in the reduced state. Iron precipitation was not a problem since iron(II) is highly soluble in aqueous solutions. Control experiments were run without specimens to insure no precipitation. In the first experiment, 4 *S. clava* specimens were placed in 600 ml of sea water with 4.4×10^{-7} *M* ^{55}Fe(II)citrate and 0.4 m*M* ascorbate. In the second experiment, two containers were set up, each containing three equal-sized specimens in 500 ml of sea water with 1.0×10^{-7} *M* $^{55}Fe\ SO_4$ and 10^{-6} *M* ascorbate. Sea water aliquots were removed periodically for up to eight hours and counted by liquid scintillation.

Iron uptake by blood cells

The blood of several *S. clava* specimens was collected in a centrifuge tube as previously described (Agudelo *et al.*, 1983). Twenty mg of Cleland's reagent were added to the whole blood to prevent cell agglutination. The blood cells were separated from the plasma by centrifuging at $1200 \times g$ for five minutes, and carefully decanting the plasma. A premeasured volume (generally 1.0 ml) of 0.5 *M* NaCl, 0.1 *M* Hepes (pH 7.3) was added to the centrifuge tubes, and the cells were resuspended by vortexing for 30 seconds at a moderate speed. The same amount of buffer was added to another centrifuge tube, as a control. The blood cell suspension and control solution were kept in an ice bath at all times. At time t = 0 an equal amount of radioactive iron(II) was added to both the cell suspension and control. Aliquots of blood cell suspension (50–100 μl) were sampled at various intervals of time (approximately every 15 s to 10 min for up to 1 h), transferred into microcentrifuge tubes and centrifuged for 15 seconds at $6500 \times g$. The supernatant was counted by liquid scintillation. An equal volume of control solution was also counted to provide the cpm for supernatant at time zero (initial iron concentration). Since the amount of blood suspension sampled was so small, it would be statistically invalid to count the number of cells in each aliquot.



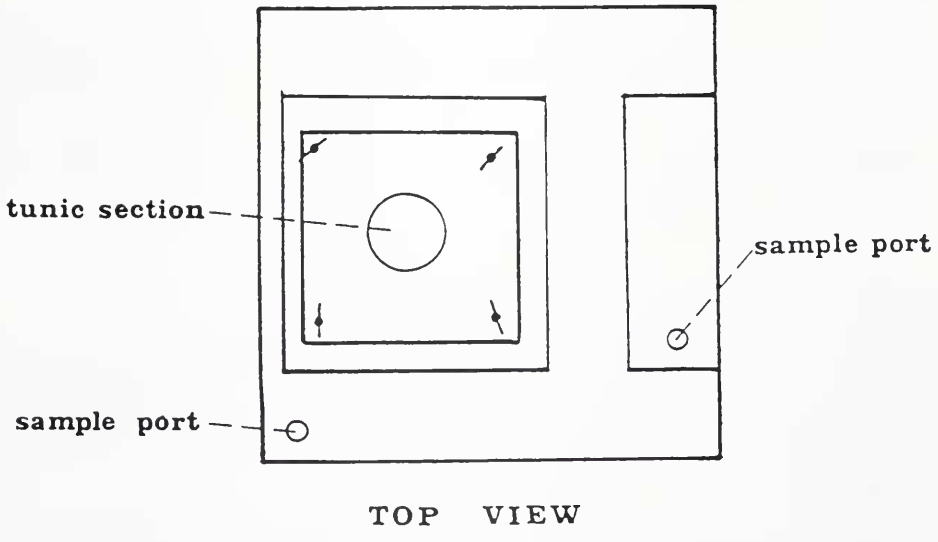


FIGURE 1. Plexiglass container used to determine iron absorption and permeability by *Styela clava* tunic in sea water.

Therefore the decline in iron concentration in the buffer solution is a better indicator of iron uptake by blood cells in suspension.

Several experiments were run to determine the relationship of iron concentration to the cell concentration in the cell suspension. In this case the blood of the specimens

was divided into two equal volumes and centrifuged to remove the plasma. One blood pellet was resuspended in buffer to measure iron uptake. The second blood pellet was analyzed for protein content using the Lowry method (Lowry *et al.*, 1951). Protein concentration was taken to be proportional to blood cell concentration.

Several metabolic inhibitors such as citric acid (1–9 m*M*), 2-deoxyglucose (5 m*M*), 4,4-paradinitro-stilbene-2,2 paradisulfonic acid, disodium salt (DNDS) (0.6 mg per ml), and phosphate (2.6 m*M*) were studied. The inhibitor was added to both blank and cell suspensions approximately 15 to 30 minutes before the addition of radioactive iron(II). The effect of an inhibitor was also studied by comparing iron uptake in treated and non-treated cell suspensions. Two blood cell suspensions, with approximately the same number of cells, were placed in an ice bath. The first cell suspension was used as a control; the inhibitor was added to the other suspension. The suspensions were incubated for fifteen minutes before adding the iron. The iron remaining in solution was measured as a function of time, for approximately one hour.

Results

Iron uptake from sea water by S. clava

Citrate concentrations up to 5 m*M* did not have any visible effect on *S. clava* since the rate of oxygen consumption before and after addition of the citrate was the same (Fig. 2). We can therefore assume that there is no acute toxic effect caused by the citrate and that our uptake studies are not affected by the 1000-fold citrate excess used to keep iron(III) from precipitating.

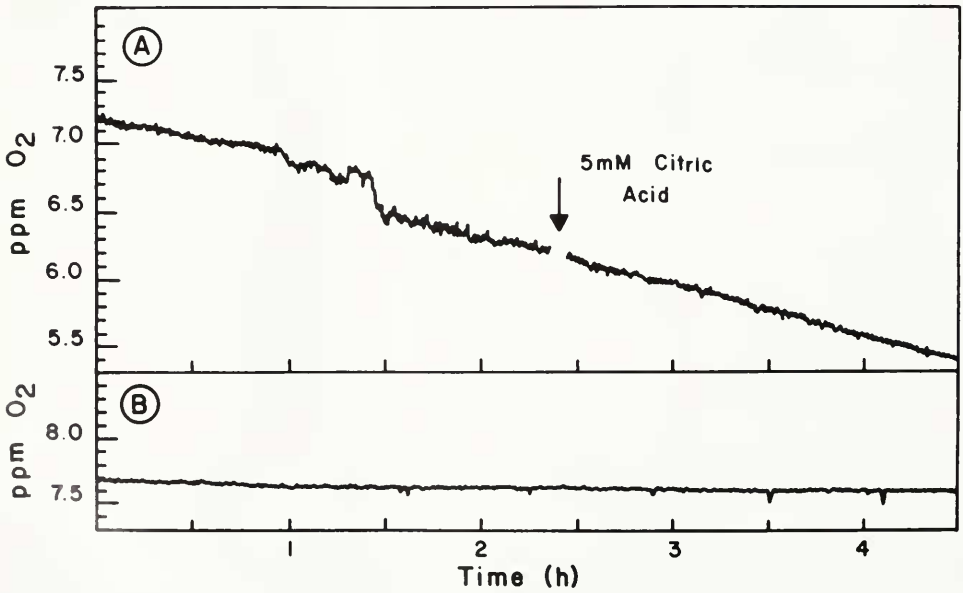


FIGURE 2. Decline in sea water oxygen over time for (A) a respiration chamber containing 5 adult *Styela clava* in 1.66 liters of 0.45 μm filtered sea water (12°C) both before and after the addition of citric acid (final concentration approximately 5 m*M*), and for (B) a respiration chamber filled with sea water alone.

Initial experiments on Fe(III) uptake by intact *S. clava* in solutions containing a 1000-fold excess of citrate demonstrated that iron concentrations in the surrounding sea water declined over time. No decrease in sea water iron concentrations was observed in containers without specimens. Semilog plots of iron cpm *versus* time gave reasonably straight lines with negative slopes for containers with *S. clava*. However, the rate of sea water iron decline varied in each experiment even though the initial iron concentration, the total amount of sea water used, and the size of the animal were approximately the same. Liquid scintillation analyses of tunic pieces dissected from ^{55}iron(III)-exposed animals, showed that the majority of the radioiron was associated with the tunic.

Experiments in which pieces of *S. clava* tunic were used as partitions between two separate chambers (Fig. 1) (one containing radioiron in seawater, the other containing clean sea water) demonstrated that tunic is impermeable to dissolved iron(III) in sea water. Although a decrease in radioactive iron was observed in the sea water exposed to the outer surface of the tunic, no radioiron was found in the sea water exposed to the inner surface of the tunic even after 48 hours of exposure. We conclude that the outer surface of the tunic is responsible for much of the reduction in sea water iron(III) concentrations observed in our previous experiments. However, when a piece of tunic was coated with Vaseline, and placed in sea water with radioiron and a 1000-fold citrate, no decrease of radioiron from solution was observed.

The precipitation of iron(III) onto the tunic was prevented by lightly coating each *S. clava* with Vaseline prior to the start of the experiments. As a result, the decline in sea water Fe(III) concentrations was much slower than observed in previous experiments. The rate of decline was entirely reproducible at 10^{-7} *M* $^{55}FeCl_3$ (Fig. 3). Although the specimen in container 1 died within the first six hours of initiating the experiment, iron measurements were continued along with the other two containers for over 36 hours. Remarkably, the rate of radioiron removal from the sea water was the same for all three containers, regardless of whether the animals were dead or alive. These results indicate that there is no active uptake.

In contrast to Fe(III), no uptake of Fe(II) was observed for *S. clava*. Sea water Fe(II)citrate and sea water Fe(II)sulfate concentrations remained constant in both control and experimental containers over the course of 4 to 24 hours.

Iron uptake by S. clava *blood cells*

Iron(II) uptake by blood cells appears to be biphasic (Fig. 4). When the log cpm is plotted *versus* time, the curve obtained can be divided into two linear portions, which we refer to as the fast and slow phases (Table I). Keeping the iron concentration constant, the iron uptake rate in the slow phase varies with the total protein content (*i.e.*, proportional to the number of cells) in the cell suspension. However the calculated rate constants for this phase are the same regardless of the number of cells in the suspension (Table I). The fast phase is not clearly defined probably because of the high concentration of iron used in all three experiments.

If the iron(II) concentration is decreased 10-fold, both the fast and slow phases can be seen more clearly. The rate constant for the slow phase is approximately the same for all experiments ($5 \times 10^{-4} \cdot s^{-1}$). It is clear that the radioiron is taken in by the tunicate blood cells.

Radiolabeled iron(II) was not simply exchanged with iron already present in the blood cells. When the cells were incubated in a medium containing $^{55}FeSO_4$, 44% of the total radioactive iron was taken up by the cell. When the remaining radioactive supernatant was removed by centrifugation and the cells resuspended in clean buffer

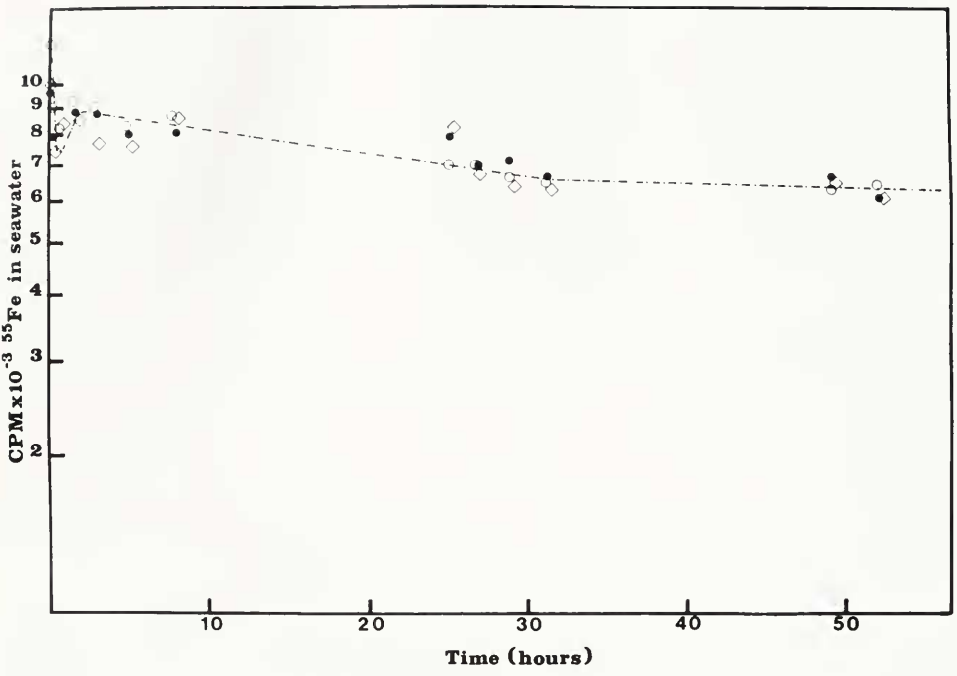


FIGURE 3. Iron uptake by *Styela clava* specimens in sea water containing 1.07×10^{-7} *M* $^{55}FeCl_3$ (specific activity 34.71 mCi mg^{-1} Fe) and 1 m*M* citrate. Container I, ◇, 1 specimen (24.88 g) in 300 ml of sea water; container II, ●, 2 specimens (32.6 g) in 500 ml of sea water; container III, ○, 2 specimens (32.3 g) in 500 ml of sea water.

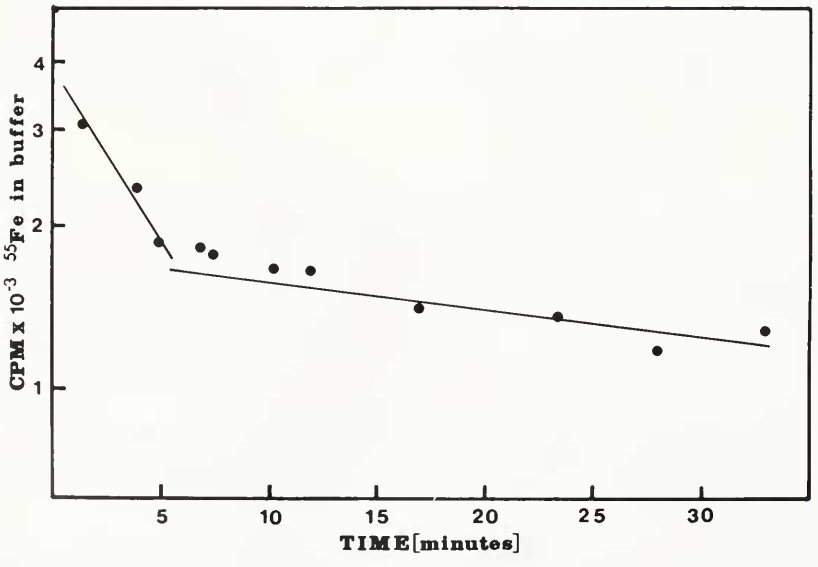


FIGURE 4. Iron(II) uptake by *Styela clava* blood cells suspended in buffer solution (0.1 *M* HEPES, pH 7.3, 0.5 *M* NaCl) containing an initial iron(II) sulfate concentration of 1.6×10^{-6}, and 3×10^{-5} *M* ascorbate. The data was fitted to an exponential function of the form: $y = B_1 + B_2 \exp[-B_3x] + B_4 \exp[-B_5x]$. The constants obtained were: $B_1 = 1130 \pm 9$ cpm, $B_2 = 2907 \pm 38$ cpm, $B_3 = 0.006 \pm 0.0001$ s^{-1}, $B_4 = 1067 \pm 8$ cpm, $B_5 = 0.00058 \pm 0.00002$ s^{-1}.

TABLE I

Iron uptake by Styela clava *blood cells*

Initial Fe(II) conc. (μM)	Total protein conc. (mg)	Rate Constant $\times 10^3 \cdot s^{-1}$ slow
22.1	7.24	0.52
22.1	14.29	0.52
22.1	21.65	0.52

solution, only 2% of the radioiron was observed in solution, probably due to the extracellular buffer solution trapped in the blood cell pellet during centrifugation. The level of radioiron in solution did not increase and therefore no leakage of radioactive iron was observed for 30 minutes. This result indicates that the iron is irreversibly taken up by the cells during the time span in which we studied uptake.

Uptake of Fe(II) was inhibited by 2–9 mM citrate (Tables II and III). Inhibition was not simply due to the drop in pH caused by the addition of citric acid, since inhibition was also observed in neutralized solution. No inhibition of Fe(II) uptake was observed with either 2-deoxyglucose, gallium(3+), phosphate, or the stilbene derivative DNDS (Tables II and III).

DISCUSSION

Iron chemistry in solution is complex, including several solubility products as a result of extensive hydrolysis (Baes and Mesmer, 1976). Iron concentrations quoted for sea water vary greatly (Chester and Stoner, 1974), partly because of the way dissolved and particulate iron are defined. Chester and Stoner (1974) report the dissolved iron concentration in sea water to be $0.4–8.6 \times 10^{-8}$ for near-shore surface waters (water within 400 km of land), having defined dissolved iron as iron that passes through a filter with pores 0.45 μm in diameter, and is retained by a CHELEX column. Betzer and Pilson (1970) report the particulate iron concentration in sea water to be about 3×10^{-9} M, using 0.45 μm filters to retain the particulate matter. These numbers are chosen because the methodologies used are clear. However, it should be noted that many bacteria can pass through 0.45 μm filters. Bacterially associated iron is therefore included in the results obtained by the methods of Chester and Stoner (1974), and Betzer and Pilson (1970).

For iron uptake studies, it is not as important to have an exact number for the total iron concentration in sea water as it is to know what iron species can be found in solution. It is presently impossible to determine either the oxidation state of the

TABLE II

Iron uptake by Styela clava *in the presence of possible inhibitors*

Initial Fe(II) conc. (μM)	Inhibitors	Rate constants $\times 10^3 \cdot s^{-1}$ Fast	Slow
1.6	none	6.0	0.58
2.36	citrate (8.7 mM)	no uptake	
2.30	DNDS (1.15 mg)	N.D.*	0.52
6.86	$H_2PO_4^-$ (2.6 mM)	N.D.	1.0

* N.D. = not determined.

TABLE III

Uptake by Styela clava *blood cells in the presence of possible inhibitors* versus *control*

Treatment	Initial Fe(II) conc. in buffer (μM)	Control cells	Treated cells
2-deoxyglucose (5 mM)	2.37	+++	+++
Ga^{3+} (1×10^{-4} M)	2.20	+++	+++
citrate (pH 6.6, 8.7 mM)	2.30	+++	—
citrate (pH 7.3, 2.9 mM)	2.35	+++	—

+++ blood cell iron uptake, — no uptake.

iron, or the major ionic species present in solution at the low concentrations of iron found in sea water. Kester *et al.* (1975) developed a chemical model based on known stability constants and chemical composition of sea water to predict chemical speciation. Based on this model at least 90% of the dissolved iron in oxygenated sea water should be found as iron(III) hydroxide ($Fe(OH)_3$). The remainder is mainly $Fe(OH)_2^+$ with a trace of $Fe(OH)^{2+}$. Since *S. clava* was collected in Boston Harbor at a depth of no more than 6 m, we can assume that oxidation state +3 predominates. However, some of the organically bound iron(III) can be photoreduced by light, creating small concentrations of Fe^{2+} (Anderson and Morel, 1982). According to Kester *et al.* (1975) any ferrous (+2) ion present in sea water at a pH of around 8.0 will be oxidized to iron(III) (95% of it in less than a minute).

Iron uptake by S. clava

There are three sources of iron available to filter-feeding marine organisms: dissolved iron in sea water, particulate inorganic iron, and iron contained in their food source (*e.g.*, phytoplankton and other microorganisms). Results of experiments described in the first section indicate that tunicates either do not actively accumulate iron from the aqueous phase of sea water either as iron(III) or iron(II) or that active iron uptake is so slow that it was not observed in 52 hours of measurements. Therefore, tunicates probably obtain iron either as particulate or colloidal iron [in which case it would be in the form of iron(III) hydroxide], or from their food source.

However it is possible that iron levels in the specimens were sufficiently high so that iron uptake was minimal. Although we attempted to study iron-starved animals, we were unable to maintain animals in synthetic sea water for more than a day. We were therefore not able to restrict the iron diet from sea water using this method.

Investigations of iron uptake in marine invertebrates generally have been limited to the molluscs, particularly the mussel *Mytilus edulis* (Hobden, 1969; Pentreath, 1973; George *et al.*, 1976; George and Coombs, 1977; Lowe and Moore, 1979) and, more recently, the abalone *Haliotis discus* (Tateda *et al.*, 1984). These investigations have centered on the uptake of particulate Fe(III) and Fe(III) organic complexes. Fe(III) uptake by *Mytilus edulis* is primarily by pinocytosis, as evidenced by light microscopical and ultrastructural studies (Fowler *et al.*, 1975; George *et al.*, 1976; George and Coombs, 1977; Lowe and Moore, 1979). This process may occur in a variety of epithelial cells (mantle, digestive gland, kidney, intestine, gill, etc.). Once taken in by pinocytosis, the iron remains within membrane-limited vesicles and may even be transferred in this form to blood cells for subsequent transport throughout the mussel's body. No free cytoplasmic iron has been identified in these epithelial cells, indicating that iron does not cross cell membranes in sufficient quantity to be measured.

The precipitation of particulate $Fe(OH)_3$ onto mucus films may be important for the subsequent uptake of iron by invertebrates. In an autoradiographic study of *Mytilus edulis* tissues, Pentreath (1973) observed that the mucus sheets covering the gills were covered with particulate $^{59}Fe(III)$, whereas ^{65}Zn, being primarily soluble in sea water, was not associated with the mucus coverings. In contrast to these findings, George *et al.* (1976) found no evidence of an iron embedded mucus sheet on *M. edulis* gills at the ultrastructural level. In addition, if the mussel gills were washed, no loss of ^{59}Fe activity was observed, indicating that the label was not extracellular. However, these investigators found that ^{59}Fe was associated with mucus within the stomach and gut. Mucus, whether associated with the gills and stomach of bivalves or lining the branchial basket of tunicates, may collect and hold particulate iron prior to ingestion and subsequent uptake.

Extensive research has been carried out on iron uptake by phytoplankton (Goldberg, 1952; Anderson and Morel, 1982). Anderson and Morel (1982) showed that phytoplankton obtain iron from the free iron concentration in sea water, and that reduction of iron(III) to iron(II) increases iron uptake. They postulated that iron in phytoplankton is probably present in the ferrous state or as an iron(III)-organic complex. They further postulated the presence of a transport protein in the cell membrane which mediates iron uptake from sea water. No conclusive evidence exists on the oxidation state of iron present in this microorganism, nor is there a detailed mechanism of iron uptake. These facts must be known before a definitive study of iron uptake by tunicates from microorganisms can be undertaken.

Blood cell iron uptake

There is still a large gap in our knowledge between iron uptake by the tunicate from sea water, and iron uptake by tunicate blood cells. We can only postulate that iron is obtained by digestion of particulate matter (colloidal iron, microorganisms) in the digestive tract, where it is solubilized into a form that will reach the blood plasma.

Because of the low iron concentration in the plasma, it is difficult to specify the oxidation state of the iron present. There are proteins in the plasma that are capable of binding iron (Hawkins *et al.*, 1980). Furthermore, iron inside the blood cells is present as iron(II), and 70% of this iron is associated with cell membranes, (Agudelo *et al.*, 1983). From these data, we can hypothesize that there may be a transferrin-like protein present in the blood cell membranes that transports iron into the cells.

Tunicate blood cells accumulate iron(II) from the suspending medium. Two steps are observed: the first is probably the rapid binding of the iron(II) to the surface membrane of the cell; the second is slower transport of iron into the cell. This is the most likely mechanism, since the first step depends on iron concentration (although not enough data has been obtained to find the relationship of rate *versus* Fe concentration), while the slow step is independent of iron concentration.

Citrate completely inhibits iron(II) uptake by blood cells. This effect is probably due to complexation of iron(II), making it unavailable to the membrane proteins of the cell. Another possibility is that citrate can enter the cytosol (probably through an anionic channel), where it can inhibit phosphofructokinase. Excess citrate leads to a decrease in the rate of glycolysis (Lehninger, 1975), thereby preventing iron uptake, if it is an active process.

2-Deoxyglucose is carried into the cell by the glucose carrier (Lehninger, 1975). However, it will slow the rate of glycolysis because it can not be metabolized like glucose. 2-Deoxyglucose would thus have a similar effect on iron(II) uptake as citrate, if the process is an active one. When added to the cell suspension, 2-deoxyglucose

showed no effect on iron(II). Therefore, we can conclude that iron(II) uptake is not an active process.

Although the uptake experiments show that the iron(II) goes into the blood cells, they do not tell us anything about whether the iron is oxidized before or during the process of entering the cell. Gallium has been used as a substitute for iron in many iron metabolism studies (Emery, 1982). Ga^{3+} and Fe^{3+} ions are very similar in size (0.062 nm and 0.064 nm, respectively) and carry the same charge. If the uptake process requires iron(III) in any step, by incubating with gallium(III) prior to the uptake studies, inhibition or a decrease in the rate should be observed since a significant number of binding sites would be occupied by gallium. That gallium does not affect iron(II) uptake implies that there is no oxidation step in the iron(II) uptake by the blood cells. This result does not preclude the possibility that iron(III) could be transported into the cells. In this case it is possible that the first step would require iron(III) being reduced to iron(II). Iron(III) uptake studies by blood cells were not addressed because of the low solubility of iron(III). At this point, due to interference by precipitation, iron(III) uptake studies would be ambiguous, and firm mechanistic conclusions could not be drawn from them.

In summary, iron taken up by *S. clava* is most likely in particulate form, or incorporated in microorganisms available as food.

Once the iron reaches the plasma, it can be accumulated by the blood cells if it is in the iron(II) form, without any steps requiring oxidation changes. Because it is inhibited by citrate, iron(II) must be in its ionic form. Iron(II) uptake is an irreversible process. No conclusions can be reached about iron(III) uptake by the blood cells.

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LITERATURE CITED

AGUDELO, M. I., K. KUSTIN, AND W. E. ROBINSON. 1982. Blood chemistry of *Boltenia ovifera*. *Comp. Biochem. Physiol.* **72A:** 161–166.

AGUDELO, M. I., K. KUSTIN, G. C. MCLEOD, W. E. ROBINSON, AND R. T. WANG. 1983. Iron accumulation in tunicate blood cells. I. Distribution and oxidation state of iron in the blood of *Boltenia ovifera*, *Styela clava*, and *Molgula manhattensis*. *Biol. Bull.* **165:** 100–109.

ANDERSON, M. A., AND F. M. M. MOREL. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* **27:** 789–813.

BAES, C. F., AND R. E. MESMER. 1976. Pp. 226–237 in *The Hydrolysis of Cations*. J. Wiley and Sons, Inc., New York.

BERG, J. R. 1982. The blood chemistry of *Ascidia ceratodes;* radiotracer studies and characterization of the yellow chromogen. Ph.D. Dissertation, University of California, Davis. 195 pp.

BETZER, P. R., AND M. E. G. PILSON. 1970. Concentration of particulate iron in Atlantic open-ocean water. *J. Mar. Res.* **28:** 251–267.

BEZKOROVAINY, A. 1980. *Biochemistry of Nonheme Iron*. Plenum Press. New York. 435 pp.

CHESTER, R., AND J. H. STONER. 1974. The distribution of zinc, nickel, manganese, cadmium, copper, and iron in some surface waters from the world ocean. *Mar. Chem.* **2:** 17–32.

DINGLEY, A. L. 1982. Vanadium in tunicate blood cells: transport and intracellular pH. Ph.D. Dissertation, Brandeis University, Waltham. 102 pp.

DINGLEY, A. L., K. KUSTIN, I. G. MACARA, AND G. C. MCLEOD. 1981. Accumulation of vanadium by tunicate blood cells occurs via a specific anion transport system. *Biochim. Biophys. Acta* **649:** 493–502.

EMERY, T. 1982. Iron metabolism in humans and plants. *Am. Sci.* **70:** 626–632.

ENDEAN, R. 1953. Discovery of iron in tunicin-forming blood cells of an ascidian. *Nature* **172:** 123.

FOWLER, B. A., D. A. WOLFE, AND W. F. HETTLER. 1975. Mercury and iron uptake by cytosomes in mantle

epithelial cells of quahog clams *Mercenaria mercenaria* exposed to mercury. *J. Fish. Res. Bd. Canada* **32:** 1767–1775.

George, S. G., and T. L. Coombs. 1977. Effects of high stability iron-complexes on the kinetics of iron accumulation and excretion in *Mytilus edulis* (L.). *J. Exp. Mar. Biol. Ecol.* **28:** 133–140.

George, S. G., B. J. S. Pirie, and T. L. Coombs. 1976. The kinetics of accumulation and excretion of ferric hydroxide in *Mytilus edulis* (L.) and its distribution in the tissues. *J. Exp. Mar. Biol. Ecol.* **23:** 71–84.

Goldberg, E. D. 1952. Iron assimilation by marine diatoms. *Biol. Bull.* **102:** 243–248.

Hawkins, C. J., P. M. Merefield, D. I. Parry, W. R. Biggs, and J. H. Swinehart. 1980. Comparative study of the blood plasma of the ascidian *Pyura stolonifera* and *Ascidia ceratodes*. *Biol. Bull.* **159:** 656–668.

Hawkins, C. J., P. Kott, D. L. Parry, and J. H. Swinehart. 1983. Vanadium content and oxidation state related to ascidian phylogeny. *Comp. Biochem. Physiol.* **76B:** 555–558.

Hobden, D. J. 1969. Iron metabolism in *Mytilus edulis*. 2. Uptake and distribution of radioactive iron. *J. Mar. Biol. Assoc. U. K.* **49:** 661–668.

Kester, D. R., R. H. Byrne, Jr., and Y. J. Liang. 1975. Redox reactions and solution complexes of iron in marine systems. Pp. 56–79 in *Marine Chemistry in the Coastal Environment*. T. M. Church, ed. ACS Symposium Series. No. 18.

Kustin, K., K. V. Ladd, and G. C. McLeod. 1975. Site and rate of vanadium accumulation in the tunicate *Ciona intestinalis*. *J. Gen. Physiol.* **65:** 315–328.

Ladd, K. V. 1974. The distribution and accumulation of vanadium with respect to the tunicate *Ciona intestinalis*. Ph.D. Dissertation, Brandeis University, Waltham, MA. 108 pp.

Lenhinger, A. L. 1975. Pp. 279–308 in *Biochemistry*, 2nd ed. Worth Publishers, Inc., New York.

Lowe, D. M., and M. N. Moore. 1979. The cytochemical distributions of zinc (ZnII) and iron (FeIII) in the common mussel, *Mytilus edulis*, and their relationship with lysosomes. *J. Mar. Biol. Assoc. U. K.* **59:** 851–858.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

Macara, I. G., G. C. McLeod, and K. Kustin. 1979. Tunichrome and metal ion accumulation in tunicate blood cells. *Comp. Biochem. Physiol.* **63B:** 299–302.

Pentreath, R. J. 1973. The accumulation from water of ^{64}Zn, ^{54}Mn, ^{58}Co, and ^{59}Fe by the mussel *Mytilus edulis*. *J. Mar. Biol. Assoc. U. K.* **53:** 127–143.

Tateda, Y., M. Nakahara, and T. Koyanagi. 1984. Accumulation of iron-59 in marine animals from different uptake route. *Bull. Japn. Soc. Sci. Fish.* **50:** 89–93.

Webb, J., and P. Chrystal. 1981. Protein binding of iron in blood plasma of the ascidian *Herdmania momus*. *Mar. Biol.* **63:** 107–112.